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VERTICAL AND HORIZONTAL RESISTANCE OF $F_{5:6}$ PROGENIES OF CAROTENOID-BIOFORTIFIED LETTUCE TO *Bremia lactucae*[1]

ANA CAROLINA PIRES JACINTO[2]*, RENATA CASTOLDI[3], GABRIEL MASCARENHAS MACIEL[3], JAIR ROCHA DO PRADO[3], HAMILTON CÉSAR DE OLIVEIRA CHARLO[4]

ABSTRACT - The use of resistant cultivars is one of the strategies for downy mildew management. The objective of this study was to evaluate the vertical and horizontal resistance of $F_{5:6}$ progenies of carotenoid-biofortified lettuce to virulence phenotypes of *Bremia lactucae* 63/63/51/00, 63/31/19/00 and 63/63/19/00. The experimental design was completely randomized and subdivided into plots that were evaluated over time. In the plots, 12 genotypes of lettuce were used, and the subplots were monitored over time (7^{th} to the 18^{th} day after inoculation). For each virulence phenotypes of *Bremia lactucae*, a separate experiment was performed with three replicates. To select resistant genotypes, plants were inoculated with distilled water, sporangia removed from infected tissues and Tween 20. The genotypes were evaluated when sporulation appeared on cotyledonary leaves of the susceptible cultivar Solaris, verifying the proportion of necrotic and sporulated plants. There was a correlation between genotypes and times for all virulence phenotypes evaluated. Genotypes UFU-189#2, UFU-206#1, UFU-215#3 and UFU-215#14 showed vertical resistance to virulence phenotypes 63/63/51/00, 63/31/19/00, and 63/63/19/00 of *B. lactucae*. Horizontal resistance levels were observed in genotype UFU 206#1 for virulence phenotypes 63/63/51/00 and 63/31/19/00; in genotype UFU-66#7 for virulent phenotype 63/31/19/00; and in genotype UFU-215#10 for virulence phenotype 63/63/19/00.

Keywords: *Lactuca sativa* (L.). Downy mildew. Non-specific resistance. Specific resistance.

RESISTÊNCIA VERTICAL E HORIZONTAL DE PROGÊNIES $F_{5:6}$ DE ALFACE BIOFORTIFICADA COM CAROTENOIDES A *Bremia lactucae*

RESUMO - O uso de cultivares de alface resistentes é uma das formas de controle do míldio. Objetivou-se avaliar as resistências vertical e horizontal de progênies $F_{5:6}$ de alface biofortificada com carotenoides aos fenótipos de virulência de *Bremia lactucae*: 63/63/51/00, 63/31/19/00 e 63/63/19/00. O delineamento experimental utilizado foi inteiramente casualizado, em parcelas subdivididas no tempo. Nas parcelas utilizaram-se os 12 genótipos de alface e nas subparcelas utilizou-se o tempo, sendo este de 7 a 18 dias após a inoculação. Para cada fenótipo de virulência de *Bremia lactucae* fez-se um experimento separado, com três repetições. Para selecionar genótipos resistentes procedeu-se a inoculação, com mistura de água destilada, esporângios retirados de tecidos infectados do hospedeiro e Tween 20. A avaliação dos genótipos iniciou-se quando houve o aparecimento da esporulação nas folhas cotiledonares da cultivar suscetível Solaris, verificando-se a proporção de plantas necrosadas e com esporulação. Houve interação entre genótipos e tempos para todos os fenótipos de virulência avaliados. Os genótipos de alface biofortificada UFU-189#2, UFU-206#1, UFU-215#3 e UFU-215#14 apresentam resistência vertical aos fenótipos de virulência 63/63/51/00, 63/31/19/00 e 63/63/19/00 de *B. lactucae.* Níveis de resistência horizontal foram observadas no genótipo UFU-206#1 para os fenótipos de virulência 63/63/51/00 e 63/31/19/00; no genótipo UFU-66#7 para o fenótipo de virulência 63/31/19/00 e no genótipo UFU-215#10 para o fenótipo de virulência 63/63/19/00 de *Bremia lactucae*.

Palavras-chave: *Lactuca sativa* (L.). Míldio. Resistência não específica. Resistência específica.

*Corresponding author
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[2]Institute of Agricultural Sciences, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil; carol.agro.ufu@gmail.com - ORCID: 0000-0001-8184-5803.
[3]Institute of Agricultural Sciences, Universidade Federal de Uberlândia, Monte Carmelo, MG, Brazil; rcastoldi@ufu.br - ORCID: 0000-0001-9406-0917, gabriel@ufu.br – ORCID: 0000-0002-3004-9134, jairrp@ufu.br – ORCID: 0000-0002-6165-4126.
[4]Department of Agronomy, Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Uberaba, MG, Brazil; hamiltoncharlo@iftm.edu.br - ORCID: 0000-0003-0663-2167.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the main leafy vegetable in Brazil, and its consumption and economic and social importance are widely spread and recognized globally. Its low production cost, combined with high nutritional value and short production cycle favor the spread of its popularity.

Despite the high nutritional value, the cultivars contain low content of carotenoids, a precursor of vitamin A. In the human body, carotenoids along with other bioactive compounds enhance the anti-inflammatory and antioxidant activity and associated immune responses, and promote human health by preventing various reactive oxygen species mediated-chronic diseases (BILAL et al., 2017; CENTELLA et al., 2017; MONEGO; ROSA; NASCIMENTO, 2017; SOSA-HERNÁNDES et al., 2018). Therefore, increasing the carotenoid content in vegetables is a strategy for biofortification.

The genetic control of carotenoids in lettuce has high heritability (84%), and it can be enhanced through classical genetic breeding, increasing the carotenoid content of cultivars (CASSETARI et al., 2015). However, there are few studies that combine biofortification of lettuce with resistance to diseases. Among the diseases, downy mildew, caused by the biotrophic oomycete *Bremia lactucae* Regel, is a disease with economic importance for cultivated lettuce worldwide. Lettuce can be infected by this pathogen at any developmental stage, from young seedlings to mature plants. Infected plants develop yellow to pale green lesions following the breakdown of the biotrophic interaction that eventually become necrotic, because of secondary pathogens. *B. lactucae* infection leads to lower marketable yield and higher harvest-related expenses incurred to remove infected leaves (SIMKO et al., 2013).

The use of fungicides is currently the best option for disease management; however, the use of resistant cultivars is a suitable alternative (PARRA; SIMKO; MICHELMORE, 2021).

Lettuce genotypes with resistance to downy mildew can be obtained (CASTOLDI et al., 2014). However, during the selection of downy mildew-resistant lines, emphasis has to be placed on vertical or specific resistance, which is monogenic and short term. Therefore, the pathogen are prone to the effect of monogenic genes (BESPALHOK; GUERRA; OLIVEIRA, 2018). Moreover, vertical resistance is effective only against some virulence phenotypes of the pathogen, reducing the amount of initial inoculum and delaying the disease progression (KIMATI et al., 2005; TOBAR-TOSSE et al., 2017). However, the use of these cultivars for a long period in the field is limited.

In contrast, the horizontal or non-specific resistance is characterized by being polygenic and more durable, because it is maintained despite the appearance of new virulence phenotypes of the pathogen (BESPALHOK; GUERRA; OLIVEIRA, 2018), resulting in a reduction in the rate of development of the disease, without significantly affecting the initial inoculum (KIMATI et al., 2005). Therefore, the strategy of combining vertical and horizontal resistance to increase the durability of resistance of lettuce cultivars against *B. lactucae* is to be considered. Hence, the objective of this study was to evaluate the vertical and horizontal resistance of $F_{5:6}$ progenies of carotenoid-biofortified lettuce to virulence phenotypes of *B. lactucae*: 63/63/51/00, 63/31/19/00 and 63/63/19/00.

MATERIAL AND METHODS

The experiment was conducted at LAGEN (Laboratory of Seed Analysis and Genetic Resources) located in the campus of the Federal University of Uberlândia, Monte Carmelo Campus, between 2017 and 2018.

The experimental design was completely randomized in split-plots, where the genotypes were used in the plots (11 progenies $F_{5:6}$ + the 'Solaris' control) and the subplots were monitored over time (7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17 and 18 days after inoculation). Solaris is a standard susceptibility cultivar to *B. lactucae*.

The $F_{5:6}$ progenies used (UFU-66#3, UFU-66#7, UFU-75#2, UFU-189#2, UFU-206#1, UFU-215#3, UFU-215#6, UFU-215#10, UFU-215#12, UFU-215#13, UFU-215#14) were from the cross between the cultivar Uberlândia 10,000 x Pira 63 (Tecnoseed®). This germplasm has been previously selected for high carotenoid content in the leaves (JACINTO et al., 2019; MACIEL et al., 2019; SOUSA et al., 2019).

The three virulence phenotypes of *B. lactucae* (63/63/51/00, 63/31/19/00 and 63/63/19/00) used were from the "Júlio de Mesquita Filho" Júlio de Mesquita Filho State University Paulista. These are the most common and important virulence phenotypes in Brazil (CASTOLDI et al., 2012; NUNES et al., 2016; FRANCO et al., 2021). For each of the three *B. lactucae* virulence phenotypes, a separate experiment was performed with three replicates.

Inoculum multiplication occurred in the susceptible cultivar Solaris, then 30 seeds of each genotype previously disinfested (using 70% alcohol for one minute and then water + chlorine, in a ratio of 1:1 for 10 min) were sown in clear plastic boxes (11 x 11 x 3.5 cm), lined with two sheets of wet germ paper, and maintained for 15 days in a Biochemical Oxygen Demand (BOD) incubation chamber at a temperature of 13 °C and 12 h photoperiod.

After multiplication, *B. lactucae* virulence

phenotypes 63/63/51/00, 63/31/19/00 and 63/63/19/00 were inoculated at a concentration of 5 x 10^4 sporangia mL^{-1} using a Pasteur-type pipette. Next, the solution was deposited on seedlings until the point of runoff, according to the method of Ilott, Durgan and Michelmore (1987), modified by Franco et al. (2021). After inoculation, the boxes were placed in a BOD incubation chamber with a temperature of 13 °C. For the first six hours, the boxes were kept in a darkroom, after which, the photoperiod was adjusted to 12 h.

The evaluation started 7 days after inoculation, when the first sporulation on the cotyledonary leaves of the susceptible cultivar Solaris occurred. The number of sporulated and necrosed plants was calculated from the 7th to the 18th day after inoculation, and the proportion of sporulated and necrosed plants was calculated using the ratio between number of sporulated plants of the total plant number in each gerbox and the number of necrosed plants of the total plant number in each gerbox, respectively.

Furthermore, *B. lactucae* disease progression was determined by the area under the disease progress curve (AUDPC) in the total evaluation period for the 11 biofortified lettuce genotypes and for the Solaris cultivar, using the formula $AUDPC = \sum[((y1 + y2)/2) \times (t2 - t1)]$, where y_1 and y_2 are two consecutive assessments of the proportion of damaged tissue at times t_1 and t_2, respectively.

The proportion of sporulated and necrotic plants of each race in the genotypes was compared using analysis of variance (ANOVA). The F test was applied at each time and the means were compared by the Scott-Knott test, at 5% significance. For all analyzes, the statistical software (R CORE TEAM, 2016) was used.

RESULTS AND DISCUSSION

Interaction genotypes and time intervals was analyzed for all virulence phenotypes using the F test at 5% significance (Tables 1 to 6).

Table 1. Mean values of the proportion of sporulated plants for *B. lactucae* virulence phenotype 63/63/51/00 obtained in the trial of twelve lettuce genotypes over 12 days of evaluation (7th to 18th day after inoculation).

Genotypes	7 th day	8 th day	9 th day	10 th day	11 th day	12 th day	13 th day	14 th day	15 th day	16 th day	17 th day	18 th day
Solaris	0.07 Ea	0.19 Da	0.23 Da	0.37 Ca	0.37 Ca	0.38 Ca	0.41 Ca	0.41 Ca	0.51 Ba	0.60 Aa	0.60 Aa	0.60 Aa
UFU-66#3	0.00 Ba	0.03 Ba	0.05 Ba	0.08 Bb	0.14 Ab	0.14 Ab	0.14 Ab	0.14 Ab	0.14 Ac	0.17 Ac	0.17 Ac	0.17 Ac
UFU-66#7	0.01 Ba	0.05 Ba	0.09 Ba	0.16 Ab	0.16 Ab	0.16 Ab	0.21 Aa	0.21 Aa	0.21 Ab	0.21 Ab	0.21 Ab	0.21 Ab
UFU-75#2	0.01 Ba	0.10 Ba	0.20 Aa	0.20 Aa	0.25 Aa	0.25 Aa	0.25 Aa	0.25 Aa	0.25 Ab	0.25 Ab	0.25 Ab	0.25 Ab
UFU-189#2	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ac
UFU-206#1	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ac
UFU-215#3	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ac
UFU-215#6	0.00 Aa	0.01 Aa	0.03 Aa	0.04 Ab	0.04 Ab	0.04 Ab	0.04 Ab	0.04 Ab	0.05 Ac	0.05 Ac	0.05 Ac	0.05 Ac
UFU-215#10	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ac
UFU-215#12	0.00 Ca	0.19 Ba	0.26 Ba	0.33 Aa	0.33 Aa	0.33 Aa	0.33 Aa	0.33 Aa	0.33 Ab	0.33 Ab	0.33 Ab	0.33 Ab
UFU-215#13	0.01 Aa	0.05 Aa	0.08 Aa	0.08 Ab	0.09 Ab	0.09 Ab	0.11 Ab	0.11 Ab	0.11 Ac	0.11 Ac	0.11 Ac	0.11 Ac
UFU-215#14	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ac

Means followed by the same capital letter in the rows and lower-case letters in the columns did not differ significantly, as determined by Scott-Knott's test at 5% significance. Upper-case letters in the lines compare the same genotype over 12 days of evaluation (from 7 to 18 days after inoculation) and lower case letters in each column compare different genotypes on each evaluation day.

Table 2. Mean values of the proportion of plants showing necrosis for *B. lactucae* virulence phenotype 63/63/51/00 obtained in the trial of twelve lettuce genotypes over 12 days of evaluation (7th to 18th day after inoculation).

Genotypes	7 th day	8 th day	9 th day	10 th day	11 th day	12 th day	13 th day	14 th day	15 th day	16 th day	17 th day	18 th day
Solaris	0.04 Ca	0.04 Ca	0.05 Ca	0.07 Cb	0.08 Cb	0.11 Cb	0.15 Bb	0.15 Bb	0.23 Ba	0.39 Aa	0.42 Aa	0.42 Aa
UFU-66#3	0.07 Ba	0.12 Ba	0.19 Ba	0.22 Aa	0.25 Aa	0.28 Aa	0.29 Aa	0.29 Aa	0.32 Aa	0.32 Aa	0.32 Aa	0.32 Aa
UFU-66#7	0.00 Ba	0.00 Ba	0.00 Ba	0.00 Bb	0.00 Bb	0.00 Bb	0.07 Ab	0.07 Ab	0.13 Ab	0.13 Ab	0.13 Ab	0.13 Ab
UFU-75#2	0.00 Ba	0.01 Ba	0.01 Ba	0.01 Bb	0.01 Bb	0.01 Bb	0.05 Bb	0.09 Bb	0.09 Bb	0.18 Ab	0.18 Ab	0.28 Aa
UFU-189#2	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.02 Ab	0.02 Ab	0.02 Ab	0.02 Ab	0.05 Ab
UFU-206#1	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.00 Ab	0.00 Ab	0.03 Ab	0.03 Ab	0.07 Ab	0.07 Ab	0.07 Ab
UFU-215#3	0.07 Aa	0.07 Aa	0.07 Aa	0.07 Ab	0.07 Ab	0.07 Ab	0.09 Ab	0.09 Ab	0.11 Ab	0.11 Ab	0.11 Ab	0.11 Ab
UFU-215#6	0.01 Ba	0.04 Ba	0.07 Ba	0.10 Ab	0.12 Ab	0.12 Ab	0.12 Ab	0.15 Ab	0.16 Ab	0.17 Ab	0.17 Ab	0.17 Ab
UFU-215#10	0.00 Ba	0.00 Ba	0.00 Ba	0.00 Bb	0.03 Bb	0.06 Bb	0.07 Bb	0.07 Bb	0.13 Ab	0.19 Ab	0.19 Ab	0.19 Ab
UFU-215#12	0.00 Ca	0.04 Ca	0.08 Ca	0.07 Cb	0.13 Cb	0.15 Cb	0.17 Bb	0.27 Ab	0.29 Aa	0.32 Aa	0.34 Aa	0.36 Aa
UFU-215#13	0.12 Da	0.12 Da	0.17 Da	0.22 Ca	0.23 Ca	0.25 Ca	0.30 Ba	0.30 Ba	0.30 Aa	0.33 Ba	0.33 Ba	0.42 Aa
UFU-215#14	0.05 Aa	0.08 Aa	0.07 Aa	0.08 Ab	0.08 Ab	0.11 Ab	0.11 Ab	0.13 Ab	0.13 Ab	0.15 Ab	0.15 Ab	0.20 Ab

Means followed by the same capital letter in the rows and lower-case letters in columns did not differ significantly, as determined by Scott-Knott's test at 5% significance. Upper-case letters in the lines compare the same genotype over 12 days of evaluation (from 7 to 18 days after inoculation) and lower-case letters in each column compare different genotypes on each evaluation day.

Table 3. Mean values of the proportion of sporulated plants for *B. lactucae* virulence phenotype 63/31/19/00 obtained in the trial of twelve lettuce genotypes over 12 days of evaluation (7th to 18th day after inoculation).

Genotypes	7 th day	8 th day	9 th day	10 th day	11 th day	12 th day	13 th day	14 th day	15 th day	16 th day	17 th day	18 th day
Solaris	0.05 Fa	0.35 Ea	0.40 Da	0.59 Ca	0.64 Ba	0.66 Ba	0.66 Ba	0.66 Ba	0.66 Ba	0.77 Aa	0.77 Aa	0.77 Aa
UFU-66#3	0.00 Ba	0.03 Bb	0.04 Bc	0.06 Bc	0.06 Bc	0.06 Bc	0.06 Bc	0.09 Ac	0.09 Ac	0.10 Ac	0.10 Ac	0.10 Ac
UFU-66#7	0.00 Aa	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.03 Ad	0.03 Ad	0.03 Ad	0.03 Ad	0.03 Ad	0.03 Ad
UFU-75#2	0.02 Fa	0.11 Eb	0.25 Db	0.29 Db	0.31 Cb	0.31 Cb	0.31 Cb	0.32 Cb	0.32 Cb	0.39 Bb	0.39 Bb	0.46 Ab
UFU-189#2	0.00 Aa	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad
UFU-206#1	0.00 Aa	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad
UFU-215#3	0.00 Aa	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad
UFU-215#6	0.00 Ba	0.01 Bb	0.04 Bc	0.05 Bc	0.06 Ac	0.08 Ac	0.08 Ac	0.08 Ac	0.09 Ac	0.11 Ac	0.11 Ac	0.11 Ac
UFU-215#10	0.00 Aa	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad
UFU-215#12	0.00 Ca	0.07 Bb	0.12 Ac	0.13Ac	0.16 Ac	0.16 Ac	0.16 Ac	0.16 Ac	0.16 Ac	0.16 Ac	0.16 Ac	0.16 Ac
UFU-215#13	0.00 Ba	0.00 Bb	0.02 Bc	0.06 Ac	0.08 Ac	0.08 Ac	0.08 Ac	0.09 Ac	0.10 Ac	0.10 Ac	0.10 Ac	0.10 Ac
UFU-215#14	0.00 Aa	0.00 Ab	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad

Means followed by the same capital letter in the rows and lower-case letters in the columns did not differ significantly, as determined by Scott-Knott's test at 5% significance. Upper-case letters in the lines compare the same genotype over 12 days of evaluation (from 7 to 18 days after inoculation) and lower-case letters in each column compare different genotypes on each evaluation day.

Table 4. Mean values of the proportion of plants showing necrosis for *B. lactucae* virulence phenotype 63/31/19/00 obtained in the trial of twelve lettuce genotypes over 12 days of evaluation (7th to 18th day after inoculation).

Genotypes	7 th day	8 th day	9 th day	10 th day	11 th day	12 th day	13 th day	14 th day	15 th day	16 th day	17 th day	18 th day
Solaris	0.05 Ea	0.05 Ea	0.05 Ea	0.10 Ea	0.19 Da	0.23 Da	0.26 Ca	0.32 Ca	0.45 Ba	0.52 Ba	0.54 Ba	0.61 Aa
UFU-66#3	0.23 Ba	0.28 Ba	0.31 Ba	0.37 Aa	0.39 Aa	0.39 Aa	0.39 Aa	0.39 Aa	0.39 Aa	0.39 Aa	0.39 Ab	0.40 Ab
UFU-66#7	0.00 Aa	0.04 Aa	0.04 Aa	0.04 Aa	0.04 Aa	0.04 Aa	0.04 Ab	0.04 Ab	0.04 Ac	0.04 Ac	0.04 Ac	0.04 Ac
UFU-75#2	0.00 Ca	0.00 Ca	0.01 Ca	0.03 Ca	0.04 Ca	0.06 Ca	0.15 Bb	0.19 Ba	0.23 Ab	0.27 Ab	0.27 Ab	0.29 Ac
UFU-189#2	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.01 Ab	0.01 Ab	0.01 Ac	0.01 Ac	0.01 Ac	0.01 Ac
UFU-206#1	0.03 Ca	0.07 Ca	0.07 Ca	0.08 Ca	0.13 Ca	0.13 Ca	0.13 Cb	0.13 Cb	0.13 Cc	0.20 Bc	0.20 Bc	0.26 Ac
UFU-215#3	0.06 Aa	0.09 Aa	0.12 Aa	0.12 Aa	0.12 Aa	0.12 Aa	0.12 Ab	0.12 Ab	0.13 Ac	0.13 Ac	0.13 Ac	0.13 Ac
UFU-215#6	0.05 Ba	0.06 Ba	0.08 Ba	0.10 Ba	0.10 Ba	0.13 Aa	0.13 Ab	0.13 Ab	0.15 Ac	0.17 Ac	0.17 Ac	0.18 Ac
UFU-215#10	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Aa	0.00 Ab	0.00 Ab	0.04 Ac	0.04 Ac	0.04 Ac	0.07 Ac
UFU-215#12	0.00 Ba	0.00 Ba	0.00 Ba	0.00 Ba	0.01 Ba	0.01 Ba	0.07 Ab	0.09 Ab	0.12 Ac	0.12 Ac	0.13 Ac	0.15 Ac
UFU-215#13	0.04 Ba	0.06 Ba	0.09 Ba	0.11 Ba	0.18 Aa	0.18 Aa	0.23 Aa	0.25 Aa	0.25 Ab	0.25 Ab	0.25 Ab	0.26 Ac
UFU-215#14	0.07 Aa	0.08 Aa	0.09 Aa	0.08 Aa	0.09 Aa	0.09 Aa	0.10 Ab	0.10 Ab	0.10 Ac	0.10 Ac	0.10 Ac	0.10 Ac

Means followed by the same capital letter in the rows and lower-case letters in the columns did not differ significantly, as determined by Scott-Knott's test at 5% significance. Upper-case letters in the lines compare the same genotype over 12 days of evaluation (from 7 to 18 days after inoculation) and lower-case letters in each column compare different genotypes on each evaluation day.

Table 5. Mean values of the proportion of sporulated plants for *B. lactucae* virulence phenotype 63/63/19/00 obtained in the trial of twelve lettuce genotypes over 12 days of evaluation (7th to 18th day after inoculation).

Genotypes	7 th day	8 th day	9 th day	10 th day	11 th day	12 th day	13 th day	14 th day	15 th day	16 th day	17 th day	18 th day
Solaris	0.05 Da	0.39 Ca	0.47 Ba	0.59 Aa	0.61 Aa	0.63 Aa	0.63 Aa	0.63 Aa	0.63 Aa	0.63 Aa	0.63 Aa	0.63 Aa
UFU-66#3	0.00 Da	0.08 Cc	0.21 Bb	0.25 Bb	0.29 Bb	0.36 Ab	0.36 Ab	0.36 Ab	0.36 Ab	0.36 Ab	0.36 Ab	0.36 Ab
UFU-66#7	0.00 Ba	0.02 Bc	0.07 Bc	0.09 Ac	0.09 Ac	0.14 Ac	0.14 Ac	0.14 Ac	0.14 Ac	0.14 Ac	0.14 Ac	0.14 Ac
UFU-75#2	0.00 Ba	0.03 Bc	0.07 Bc	0.12 Ac	0.13 Ac	0.13 Ac	0.16 Ac	0.16 Ac	0.16 Ac	0.18 Ac	0.18 Ac	0.18 Ac
UFU-189#2	0.00 Aa	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad
UFU-206#1	0.00 Aa	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad
UFU-215#3	0.00 Aa	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad
UFU-215#6	0.00 Da	0.04 Dc	0.14 Cc	0.15 Cc	0.16 Cc	0.19 Bc	0.20 Bc	0.22 Ab	0.22 Ad	0.22 Bc	0.22 Bc	0.22 Bc
UFU-215#10	0.00 Aa	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 d
UFU-215#12	0.00 Da	0.15 Cb	0.25 Bb	0.35 Ab	0.38 Ab	0.38 Ab	0.38 Ab	0.41 Ab	0.41 Ab	0.41 Ab	0.41 Ab	0.41 Ab
UFU-215#13	0.00 Ba	0.02 Bc	0.01 Bc	0.18 Ac	0.21A c	0.22 Ac	0.23 Ac	0.23 Ac	0.23 Ac	0.23 Ac	0.23 Ac	0.23 Ac
UFU-215#14	0.00 Aa	0.00 Ac	0.00 Ac	0.00 Ac	0.00 Ad	0.00 Ac	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad	0.00 Ad

Means followed by the same capital letter in the rows and lowercase letters in the columns did not differ significantly, as determined by Scott-Knott's test at 5% significance. Upper-case letters in the lines compare the same genotype over 12 days of evaluation (from 7 to 18 days after inoculation) and lower-case letters in each column compare different genotypes on each evaluation day.

Table 6. Mean values of the proportion of plants showing necrosis for *B. lactucae* virulence phenotype 63/63/19/00, obtained in the trial of twelve lettuce genotypes over 12 days of evaluation (7th to 18th day after inoculation).

Genotypes	7 th day	8 th day	9 th day	10 th day	11 th day	12 th day	13 th day	14 th day	15 th day	16 th day	17 th day	18 th day
Solaris	0.05 Ea	0.07 Ea	0.07 Ea	0.09 Eb	0.26 Db	0.33 Ca	0.40 Ca	0.58 Ba	0.72 Aa	0.74 Aa	0.74 Aa	0.78 Aa
UFU-66#3	0.29 Ca	0.33 Ca	0.37 Ca	0.50 Ba	0.50 Ba	0.54 Ba	0.55 Ba	0.58 Aa	0.58 Aa	0.65 Aa	0.69 Aa	0.70 Aa
UFU-66#7	0.00 Ba	0.00 Ba	0.00 Ba	0.05 Bb	0.05 Bb	0.14 Ab	0.14 Ab	0.14 Ab	0.17 Ac	0.17 Ac	0.17 Ac	0.17 Ac
UFU-75#2	0.00 Ba	0.00 Ba	0.01 Ba	0.01 Bb	0.01 Bb	0.03 Bb	0.03 Bb	0.04 Bb	0.04 Bc	0.13 Ac	0.15 Ac	0.21 Ac
UFU-189#2	0.00 Aa	0.03 Aa	0.03 Aa	0.05 Ab	0.05 Ab	0.08 Ab	0.08 Ab	0.09 Ab	0.09 Ac	0.12 Ac	0.12 Ac	0.19 Ac
UFU-206#1	0.04 Ba	0.06 Ba	0.06 Ba	0.09 Bb	0.09 Bb	0.09 Bb	0.13 Bb	0.13 Bb	0.13 Bc	0.17 Bc	0.22 Ac	0.31 Ac
UFU-215#3	0.03 Aa	0.04 Aa	0.04 Aa	0.07 Ab	0.07 Ab	0.10 Ab	0.11 Ab	0.11 Ab	0.11 Ac	0.11 Ac	0.11 Ac	0.11 Ac
UFU-215#6	0.16 Ba	0.19 Ba	0.21 Ba	0.23 Bb	0.38 Ab	0.41 Aa	0.42 Aa	0.45 Aa	0.48 Ab	0.48 Ab	0.48 Ab	0.48 Ab
UFU-215#10	0.00 Aa	0.01 Aa	0.01 Aa	0.01 Ab	0.01 Ab	0.01 Ab	0.01 Ab	0.03 Ab	0.03 Ac	0.03 Ac	0.03 Ac	0.03 Ac
UFU-215#12	0.00 Ba	0.01 Ba	0.03 Ba	0.03 Bb	0.03 Bb	0.04 Bb	0.11 Bb	0.23 Ab	0.23 Ac	0.23 Ac	0.26 Ac	0.33 Ac
UFU-215#13	0.10 Ba	0.12 Ba	0.12 Ba	0.16 Bb	0.16 Bb	0.23 Bb	0.30 Aa	0.34 Ab	0.38 Ab	0.39 Ab	0.39 Ac	0.43 Ab
UFU-215#14	0.09 Aa	0.12 Aa	0.16 Aa	0.17 Ab	0.17 Ab	0.20 Ab	0.21 b	0.25 Ab	0.26 Ac	0.27 Ac	0.27 Ac	0.33 Ac

Means followed by the same capital letter in the rows and lower-case letters in the columns did not differ significantly, as determined by Scott-Knott's test at 5% significance. Upper-case letters in the lines compare the same genotype over 12 days of evaluation (from 7 to 18 days after inoculation) and lower-case letters in each column compare different genotypes on each evaluation day.

The necrosis in these genotypes can be explained through the hypersensitive response (HR) of the plant and not necessarily the infection of the pathogen. The HR is considered as one of the main events of the defense response of the plant against pathogen attack, characterized by a rapid and localized response that occurs at the site of infection of the pathogen. Among the main characteristics of the response are the rapid and localized collapse of the plant tissue around the infection site, caused by the release of toxic compounds, which also act in some cases directly on the pathogen (BALINT-KURTI, 2019).

Analyzing the interaction between genotypes and incubation time for all evaluated virulence phenotypes of *B. lactucae* (63/63/51/00, 63/31/19/00 and 63/63/19/00), it was verified that for the proportion of sporulated plants, there was statistical difference between genotypes and the susceptible cultivar Solaris, from 10, 9 and 8 days after inoculation, respectively, for virulence phenotypes 63/63/51/00, 63/31/19/00 and 63/63/19/00 of *B. lactucae*. However, genotypes UFU-189#2, UFU-206#1, UFU-215#3, UFU-215#10 and UFU-215#14 did not show a significant increase in the proportion of sporulated plants, regardless of the virulence phenotype (Tables 1, 3 and 5).

Furthermore, for the proportion of necrotic plants (virulence phenotypes 63/63/51/00, 63/31/19/00 and 63/63/19/00 of *B. lactucae*), there was a statistical difference between genotypes and the susceptible cultivar Solaris 10 and 13 days after inoculation, respectively for virulence phenotypes 63/63/51/00, 63/31/19/00 and 63/63/19/00 of *B. lactucae*. However, genotypes UFU-189#2, UFU-215#3 and UFU-215#14 did not show a significant increase in the proportion of necrotic plants, regardless of the virulence phenotype (Tables 2, 4 and 6).

Regardless of the race evaluated, the average of the proportion of both sporulated and necrotic plants of genotypes UFU-189#2, UFU-215#3, UFU-215#6 and UFU-215#14 remained statistically the same throughout the evaluation days, showing a lack of evolution of the disease, which is an indication of vertical resistance. The absence of necrotic plants and sporulated plants in the $F_{5:6}$ generation indicates the possibility of genetic control, because of a single gene with complete dominance effect (ARAÚJO et al., 2014; JACINTO et al., 2019). Probably this resistance is caused by the presence of one or more Dm gene (s) of resistance in the genotype, because most of the identified genes confer high levels of resistance. Over thirty race-specific, single dominant genes or resistance factors (Dm or R factors) have been identified in lettuce (SIMKO et al., 2013).

Some isolates that are present in one area can infect lettuce cultivars from another area, even if they have resistance genes (MAISONNEUVE, 2011). This may be because of the fact that several virulence phenotypes of *B. lactucae* have been identified so far in Brazil (NUNES et al., 2016).

The IBEB committee identified about 24 Dm genes and R factors (ISF, 2018), which confer resistance to lettuce cultivars. Although these genes provide high levels of resistance, they only remain effective for limited periods because of changes in pathogenic virulence (MICHELMORE; WONG, 2008). Therefore, new strategies are to be developed to provide more durable forms of resistance to the numerous developed lettuce cultivars.

Only genotypes UFU-66#3, UFU-75#2 and UFU-215#12 showed an increase in the proportion of sporulated and necrotic plants during the evaluation for all *B. lactucae* virulence phenotypes (Tables 1 to 6). Such an increase may indicate the presence of the DMR6 gene (STASSEN et al., 2012; ZEILMAKER et al., 2015). Lettuce cultivars that expressed such a gene may show an increase in the gene expression for susceptibility to *B. lactucae*.

However, natural variations in DMR6 conferring resistance to *B. lactucae* have not yet been identified (PARRA et al., 2016).

Sporulation did not occur in genotype UFU-215#10, when inoculated with virulent phenotype 63/63/51/00 (Table 1); and in genotype UFU-206#1, when inoculated with virulence phenotypes 63/31/19/00 and 63/63/19/00 (Tables 3 and 5); however, they presented symptoms of necrosis throughout the evaluation days (Tables 2, 4 and 6). This may have occurred because the time sequence between host cell necrosis and inhibition of fungus growth varies between different cultivars and virulence phenotypes. In some host-parasite systems, fungal inhibition occurs within a few hours before necrosis; in other systems, the two events are simultaneous, and in others, inhibition of the fungus occurs a few hours after necrosis (MATIELLO; BARBIERI; CARVALHO, 1997; BALINT-KURTI, 2019).

Despite the low incidence of sporulation and/or necrosis in lettuce $F_{5:6}$ progenies, compared to the susceptible cultivar Solaris, under ideal conditions for mildew appearance, such as high air humidity and low wind speed (FALL; VAN DER HEYDEN; CARISSE, 2016), the number of sporulated and/or necrotic leaves may increase considerably, as the development of lettuce mildew is strongly related to environmental conditions (FALL et al., 2015). This implies that even plants that exhibit low sporulation and/or necrosis may become commercially infeasible, because to be accepted by the consumer market, they need to be free of symptoms and signs of disease (FALL; VAN DER HEYDEN; CARISSE, 2016).

Furthermore, among eleven evaluated biofortified genotypes, genotypes UFU-189#2, UFU-206#1, UFU-215#3 and UFU-215#14 presented vertical resistance to virulence phenotypes 63/63/51/00, 63/31/19/00 and 63/63/19/00 of *B. lactucae.* This is due to the presence of necrosis and absence of sporulation that was observed during the evaluation period (Tables 1 to 6). Therefore, such genotypes may have few genes, such as Dm-6 and R-18, which confer incomplete resistance (MICHELMORE; WONG, 2008). The DM (Downy mildew) genes or resistance factors (FR) have been widely used in lettuce cultivars, providing a high level of resistance to mildew (CASTOLDI et al., 2014).

The AUDPC proves the vertical resistance of the UFU-189#2; UFU-206#1; UFU-215#3 and UFU-215#14 genotypes for the three *B. lactucae* phenotypes evaluated, because of the lack of disease progression in these genotypes (Figure 1). The UFU-215#10 genotype considered to be horizontally resistant, presented AUDPC values similar to the genotypes with vertical resistance (Figure 1), indicating that it is a genotype with high tolerance, because it prevented the progress of disease manifestation for the three evaluated phenotypes of *B. lactucae*.

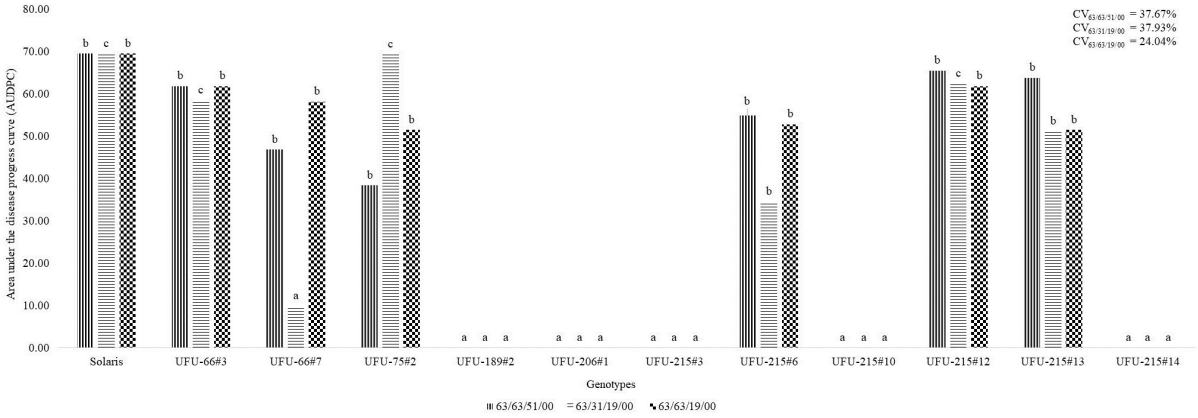

Figure 1. Disease progression of *Bremia lactucae*, demonstrated by the area under the disease progress curve (AUDPC) on total assessment period for 11 biofortified lettuce genotypes and the Solaris cultivar. Lower case letter in bars compare the different genotypes to each virulence phenotype.

CONCLUSION

Genotypes UFU-189#2; UFU-206#1; UFU-215#3 and UFU-215#14 showed vertical resistance to virulence phenotypes 63/63/51/00, 63/31/19/00 and 63/63/19/00 of *B. lactucae.* Horizontal resistance levels were observed in genotype UFU-206#1 for virulence phenotypes 63/63/51/00 and 63/31/19/00, in genotype UFU-66#7 for virulence phenotype 63/31/19/00, and in genotype UFU-215#10 for virulence phenotype 63/63/19/00 of *B. lactucae*.

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